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**ESTIMATION OF VITAMIN D AND CALCIUM LEVELS IN
CHILDREN WITH DIABETES**

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**BY
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ESTIMATION OF VITAMIN D AND CALCIUM LEVELS IN CHILDREN WITH
DIABETES

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January 2023

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ABSTRACT

ESTIMATION OF VITAMIN D AND CALCIUM LEVELS IN CHILDREN WITH DIABETES

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Master of Science in Chemistry

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The goal of this research is to the estimation of vitamin D and calcium levels in children with and without diabetes disease, evaluation of some biochemical tests and study the effect of gender and weight on study parameters and study the correlations between these parameters in diabetes children patients group and healthy individuals control groups. The results revealed that there is an essential difference in gender parameters of the two study groups, whereas it seems that the males were more sensitive to diabetes than the females. The outcomes indicated no substantial change in weight and age of the tested groups, The results of the study of the estimation of vitamin levels showed that levels of vitamin D were correlated with diabetes in children diabetes reduced levels of vitamin D in the patients. The results pointed out that the patient group significantly excelled in Ca concentration as compared to a healthy control group. The results indicated significant differences in the mean PTH level between the patient and control groups, the patient group showed significant elevation in PTH level. The study of the effect of urea level on the development of children's diabetes revealed that urea level was significantly elevated in the patient group as compared to the healthy control group. The finding appeared that the patient group significantly excelled in alkaline phosphatase levels in the blood serum of patients with diabetes as compared to healthy persons.

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Keywords: Diabetic, Vitamin D, Calcium, Insulin

ÖZET

DIYABETLİ ÇOCUKLARDA D VİTAMİNİ VE KALSIYUM DÜZEYLERİNİN TAHMİNİ

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Bu çalışmanın amacı diyabet hastalığı olan ve olmayan çocuklarda D vitamini ve kalsiyum düzeylerinin tahmin edilmesi, bazı biyokimyasal testlerin değerlendirilmesi, cinsiyet ve kilonun çalışma parametrelerine etkisinin araştırılması ve diyabetli çocuk hastalarda bu parametreler arasındaki korelasyonların araştırılmasıdır. grup ve sağlıklı bireyler kontrol gruplarıdır. Sonuçlar, iki çalışma grubunun cinsiyet parametrelerinde anlamlı bir farklılık olduğunu ortaya koyarken, erkeklerin kadınlara göre diyabete daha duyarlı olduğu görülmektedir. Sonuçlar, test edilen grupların ağırlık ve yaşlarında anlamlı bir fark olmadığını gösterdi, Vitamin düzeylerinin tahmin edilmesine yönelik çalışmanın sonuçları, çocuklarda D vitamini düzeylerinin diyabetle ilişkili olduğunu gösterdi diyabet, hastalarda D vitamini düzeylerini azalttı . Sonuçlar, hasta grubunun sağlıklı bir kontrol grubuyla karşılaştırıldığında Ca konsantrasyonunda önemli ölçüde üstün olduğuna işaret etti. Sonuçlar, hasta ve kontrol grupları arasında ortalama PTH düzeyinde önemli farklılıklar gösterdi, hasta grubu PTH düzeyinde önemli yükselme gösterdi. Üre düzeyinin çocuklarda diyabet gelişimi üzerindeki etkisinin incelenmesi, hasta grubunda üre düzeyinin sağlıklı kontrol grubuna göre önemli ölçüde yükseldiğini ortaya koydu. Bulgu, hasta grubunun diyabetli hastaların kan serumundaki alkalın fosfataz düzeylerinde sağlıklı kişilerle karşılaştırıldığında önemli ölçüde üstün olduğu ortaya çıktı.

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Anahtar Kelimeler: Diyabetik, D vitamini, Kalsiyum, İnsülin

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LIST OF SYMBOLS

-	Minus
%	Percent
**	Significant
/	Divide
+	Plus
<	Greater than
=	Equal
>	Less than
±	Plus minus
≤	Greater or equal to
≥	Less or equal to
Ca	Calcium
Cu	Copper
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m ²	Square meter
µg	Microgram
Mg	Magnesium
mg	Microgram
mIU	Milli-international units
min	Minute
mL	Milliliter
mmol	Millimole
mol	Mole
ng	Nanogram
nm	Nanometer
NS	Non-significant
rpm	Revolutions per minute
Se	Selenium
Zn	Zinc

LIST OF ABBREVIATIONS

CaSR	Calcium sensing receptors
DM	Diabetes mellitus
ECF	Extracellular fluid
FGF	Fibroblast growth factor
GDM	Gestational diabetes mellitus
HNF-4a	Hepatocyte nuclear factor 4a
ICF	Intracellular fluid
PTH	Parathyroid hormone
RBC	Red blood cells
T1DM	Type 1 diabetes mellitus
T2DM	T 2 diabetes mellitus
UV	Ultraviolet
VDR	Vitamin D receptor

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1. INTRODUCTION

Chronic diabetes, associated with significant morbidity, high mortality, and rapidly accelerating health care costs, is spreading rapidly throughout the world. By the year 2050, it is anticipated that there will be 366 million individuals living with diabetes throughout the globe, compared to 171 million in the year two thousand (Wild *et al.* 2004). Although type 2 diabetes is responsible for the majority of type 1 diabetes is increasingly being detected in new patients. Diabetes is also becoming more widespread, underscoring the need for innovative management and preventive strategies to prevent the disease. Epidemiological evidence suggests that nine out of ten cases of type 2 diabetes may have preventable or treatable causes. Routine and lifestyle (Hu *et al.* 2004).

However, long-term lifestyle modifications are difficult to implement and maintain. Modifiable risk factors for type 1 diabetes are not well understood. To avoid both forms of diabetes, it is essential to examine modifiable risk factors. Research in animals and humans has recently shown more and more evidence that vitamin D may be involved in altering diabetes risk (Pittas *et al.* 2007).

The human body may get vitamin D in one of two ways: by the consumption of foods that contain it, or through the natural production of vitamin D by the skin when exposed to UV light. This latter substance may transform into vitamin D₃ when subjected to high temperatures. The consumption of food is how the body obtains vitamin D₂. In addition to this, vitamin D₃ may enter the body of a person via the consumption of food that has been fortified with additional nutrients, as well as through the use of dietary supplements. Vitamins-D₃ (cholecalciferol) and D₂ (ergocalciferol) and differ only in the DV structure of the side chain. The differences do not affect metabolism, both forms of vitamin D act as hormones. Vitamin-D does not become biologically active until it undergoes two enzymatic transformations into the hydroxyl form. This initially occurs in the liver and is mediated by 25-hydroxylase (Cytochrome P450 2R1 [CYP2R1]) leading to the formation of 25-hydroxyvitamin D-25 (OH)D. It is metabolized in various tissues and cells of the body, and promotes the synthesis of interleukins and cytokines, as well as cathelicidin D, an antimicrobial polypeptide in macrophages (Holick 2009, Verstuyf *et al.* 2010).

Recently, the physiology of vitamin D metabolism and production has been well-reviewed (Holick 2006a, DeLuca 2004). Vitamins D2 and D3 are included under the umbrella name "vitamin D." Vitamin D is obtained by humans either by endogenous production, dietary sources, and supplementation. Fatty fish and foods fortified with vitamin D2 or vitamin D3 are good sources of vitamin D in the diet, notably fortified dairy products, baby formula, and breakfast cereals (Calvo and Barton 2004). Absorption of ultraviolet B rays by 7-dehydrocholesterol in the skin is the first and most important step in the natural synthesis of vitamin D. This stage results in the formation of vitamin D3, which is immediately converted into vitamin D3 (Holick 2006). UVB photon penetration into the epidermis, which is crucial to this process, is significantly decreased by the presence of dark skin pigmentation, sunscreen, the wintertime, or high latitudes (Holick 2005, Hollis and Wagner 2006).

The deficiency is a risk factor that has been hypothesized to be linked to T2D that has mainly been studied through human observational studies. The majority of the body's calcium, or over 99%, is found in the skeleton as the intricate calcium phosphate molecule hydroxyapatite. The bones' ability to support movement is strengthened and rigidified by this mineral, which also serves as a depot for the blood's calcium levels. Parathyroid hormone is a complex homologous hormone system composed of 1,25-dihydroxyvitamin D and ionized calcium, and trophic receptors is what regulates calcium transport in several organs (Peacock 2010). Calcium in the body is exclusively obtained through diet. Osteoporotic fractures and decreased bone mass are two symptoms of calcium deficiency. Rickets develops in youngsters who are growing fast due to a lack of calcium (Forouhi *et al.* 2012). Several studies (researcher) have linked low vit.D levels to an increased risk factor of diabetes or glucose intolerance. Several theories link the pathophysiology of type two diabetes and the development of insulin resistance to (Vit.D). Vit.D may accomplish this by modifying beta cell function, glycemic management, or both (Forouhi *et al.* 2012, Chiu *et al.* 2004, Deleskog *et al.* 2012), amongst other things. More and more data suggest that persons who have type 1 diabetes have lower levels of vit.D generally, especially when they are initially diagnosed (Borkar *et al.* 2010).

1.1 Aim of Study

The study's objective is to the estimation of vitamin D and calcium levels in children with and without diabetes disease. Evaluation of some biochemical tests and study the effect of gender and weight on study parameters and study the correlations between these parameters in diabetes children patients' group and healthy individuals control groups.



2. LITERATURE REVIEW

2.1 Diabetes

A chronic illness that is quickly spreading over the globe is linked with severe morbidity, increased mortality, and increased expenses associated with health care. It is anticipated that there will be 366 million diabetics living in the globe by the year 2050, which is an increase from 171 million in the year 2000 (Wild *et al.* 2004). Even though type 2 diabetes accounts for the vast majority of newly diagnosed cases, type 1 diabetes is on the rise. Also, diabetes is becoming more widespread, underscoring the need for innovative management and preventive strategies to prevent the disease. Epidemiological evidence suggests that nine out of ten cases of type two diabetes may have preventable or treatable causes, routines and lifestyle (Hu *et al.* 2004).

However, long-term lifestyle modifications are difficult to implement and maintain. Modifiable risk factors for type 1 diabetes are not well understood. To avoid both forms of diabetes, it is necessary to identify modifiable risk factors. Research in animals and humans has recently shown more and more evidence that vitamin D may be involved in altering diabetes risk (Pittas *et al.* 2007).

2.2 Classification of Diabetes Mellitus

2.2.1 Gestational diabetes

In general, diabetes that is generated through pregnancy and then goes away towards the conclusion of the pregnancy is known as gestational diabetes mellitus (GDM). When pregnant throughout the gestational period, females have significant glucose fluctuation and frequent rapid starvation will occur. This together with a rise in the body's production of insulin. By the conclusion of the first trimester, there is a combination of the placenta and a reduction in insulin sensitivity, insulin resistance in a transitory condition. Although this kind of diabetes cures at the end of gestation, problems can arise that may be

permanent. For instance, gestational diabetes is a possible teratogen and significantly raises the risk of death in both the mother and the fetus. Pre-eclampsia, which is linked to several anomalies in the development of the fetus, including intrauterine growth retardation (IUGR), early delivery, and stillbirth, can result from the development of diabetic nephropathy in GDM (de Souza Rugolo and Trindade 2011).

2.2.2 Maturity onset diabetes of the young

Monogenic diabetes is also known as juvenile-onset hyperglycemia (MODY) (Fajans and Polonsky 2001). Although it only affects a small percentage of diabetic patients and is typically diagnosed through the patient's second decade of life, it is still considered to be a disease. NEURODI are only some of the genes that have been linked to the development of the six different kinds (1-6) of MODY. Other genes include insulin promoter factor-I (IPF-I). HNF-J gene mutations are the most frequent. Nearly 70% of individuals with MODY have this mutation. The two most prevalent forms of the condition are MODY 2 and MODY 3. MODY 3 is distinguished by significant impairment in insulin secretion. Despite its rarity, it is crucial to correctly diagnose MODY and identify the source of diabetes to administer the best possible care. Hyperthyroidism, Cushing's disease, Klinefelters syndrome, and pancreatitis are all known to cause other secondary kinds of diabetes. Other substances that might induce secondary diabetes include thiazide diuretics, 3-blockers, calcineurin, and protease inhibitors.

2.2.3 Type 1 diabetes mellitus

Type one diabetes mellitus (D-1) is a chronic disease as a result of an autoimmune process, the beta cells of the pancreatic islets are destroyed. Insulin deficiency that requires replacement psychiatric treatment. Thus the inheritance of some HLA haplotypes is known (Human leukocyte antigens) more than half of the known genetic factors for the development of DM1(Pociot and Lernmark 2016).

However, initiation the autoimmune process of β -cells, in addition to genetic predisposition, the influence of environmental factors. Considering, the incidence of DM1 has been increasing rapidly in recent decades, which cannot be fully explained by genetic factors due to many turning slower, especially the important study of external trigger factors environment. Among the external factors provoking the development of islet autoimmunity, the most active are discusses viral infections (particularly entero viruses), reduced diversity of the intestinal microbiota, and nutritional factors: breastfeeding, the age of introducing certain food groups into the diet, especially cereals and cow's milk, a large amount of meat in the diet (Rewers and Ludvigsson 2016).

2.2.4 Type two diabetes

Diabetes type 2 is distinguished by insufficient insulin synthesis and secretion, which is brought on by insulin resistance. It's estimated that this factor is responsible for about 90 percent of all occurrences of diabetes worldwide, and diagnosis often takes place in people's forties. With increasing age, type 2 diabetes has been demonstrated to occur more frequently and more frequently. Diabetes type 2 is subdivided into two subgroups: those who are obese and those who are not fat. Patients with type 2 diabetes who are obese often develop resistance to the insulin produced by their bodies, which is linked to the location of fat storage in the tummy. This opposition is caused by changes in the cell receptors. There is a deficit in insulin production and release, as well as some insulin sensitivity at the post-receptor levels, among those who have type 2 diabetes but are not fat. Because obesity and other metabolic factors are the primary causes of T1DM, observed changes in diet and lifestyle in emerging regions have led to the greatest diabetes prevalence, which is evident in these countries (Verstuyf *et al.* 2010).

2.3 Vitamin D

Vitamin D enters the body in two ways: through food or synthesis in the skin of human under the influence of ultraviolet (UV) radiation. 7-Dihydrocholesterol is converted to provitamin D3 in the skin during sun exposure. It can also enter the body in the form of rich food or dietary supplements. This difference does not affect metabolism and both

forms of vitamin D act as hormones. Vitamin D is not biologically active until it undergoes two enzymatic reactions in the form of hydroxylation. The first of these reactions occurs in the liver and is mediated by an enzyme called 25-hydroxylase (cytochrome P450 2R1 [CYP2R1]). This reaction catalyzes the synthesis of 25-hydroxyvitamin D also known as 25(OH)D. An antibacterial polypeptide produced by macrophages along with cathelicidin D induces the synthesis of inflammatory cytokines and cytokines. It is metabolized in various tissues humans and cells of the body and plays a role in regulating proliferation and differentiation (Holick 2009, Verstuyf *et al.* 2010).

Calcitriol also known as 1,25-dihydroxyvitamin D or 1,25(OH)₂D is the product of a second reaction in the kidney with the help of an enzyme called 1-hydroxylase (CYP27B1). Calcitriol is responsible for interacting with vitamin D receptors (VDR) in cells (Holick 2009).

Because it is such an essential part of the mineral metabolism, 1,25-(OH)-2D is responsible for maintaining the correct proportions of bone metabolism. Calcium and phosphorus are two elements that are metabolized in the response to the interaction of 1,25-(OH)₂-D with the V.D.R found in the small intestine and osteoblasts. Because of the interactions that vitamin D has with VDR, the intestinal calcium absorption rate may be increased by 30–40%, and the phosphorus absorption rate can be increased by nearly 80% (Holick 2007).

If an individual does not consume an adequate amount of calcium and phosphorus, there will be a reduction in the mineralization of (Mineralisation refers or known to a process where an inorganic (molecular) substance precipitates in an organic matrix) the collagen matrix found in their bones. This will lead to the onset of rickets in kids and osteomalacia in adults. Therefore, the implications of a lack of 1,25(OH)₂D include stunted development, skeletal deformities in children, and osteoarthritis in adults (Holick 2007, Holick 2006).

Endocrine organs (including the pituitary, pancreas, parathyroid, and gonads), as well as the placenta and tissues of the cardiovascular system, and cardiomyocytes, were shown

to have VDR (Verstuyf *et al.* 2010, Temmerman 2011). The widespread presence of vitamin D receptors (VDR) in the tissues of different bodily systems is evidence that vitamin D plays a significant role in the development of a broad variety of illnesses and pathological states.

2.3.1 Vitamin D sources

In the liver of huma, vitamin D is- converted into 25-hydroxyvitamin D (25(OH)D), which is the active metabolite of vitamin D and a prohormone. 1,25-dihydroxy vitamin D (1,25(OH)2D), which plays a crucial role in the balance of calcium (Ca) and phosphorus, is the active metabolite of vitamin D. A thorough article that outlines the biology of vitamin D synthesis and metabolism has recently been published (Holick 2005, DeLuca 2004).

The vitamins D2 and D3 are included under the umbrella name "vitamin D." Vitamin D is obtained by humans either by endogenous production, dietary sources, and supplementation. Fatty fish and foods fortified with D2 or D3 are good sources of it in the diet, notably fortified dairy products, baby formula, and breakfast cereals (Calvo and Barton 2004).

The absorbance of ultraviolet B light by 7-d-cholesterol in the epidermal is the first and most essential step in the endogenous synthesis of D. This stage results in the formation of vitamin D3, which is immediately converted into vitamin D3 (Holick 2006). UVB photon penetration into the epidermis, which is crucial to this process, is significantly decreased by the presence of dark skin pigmentation, sunscreen, the wintertime, or high latitudes (Holick 2006, Hollis and Wagner 2006).

2.3.2 Vitamin D metabolism

Both vitamin D3 and vitamin D2 have important biological roles. For them to exert their biological activity and be able to operate on somatic cells, they need to undergo a very

precise metabolic process in the skin, liver, and kidneys. 7-dehydrocholesterol is transformed by the skin into vitamin-D₃ (Figure 2.1), which is subsequently produced and absorbed by the circulatory system. Vitamins D₃ and D₂ are both produced at the same time (Yu *et al.* 2019). From the lumens of the gut, where meals and nutraceuticals are absorbed, as well as from other sources. The liver is in charge of absorbing all of the vitamin-D that is circulating in the serum (bloodstream) and converting it to a metabolite called 25-(OH)-D. After that, it gets into the circulating Vit-D in the human kidneys, where it is changed and converted into a physiologically active compound. Calcitriol is the name often given to the metabolite 1,25-(OH)-2D. It is suggested to restrict serum Vit-D when assessing vitamin-D status in clinical practice for many reasons. One of these reasons is that its duration is longer compared to 1,25-(OH)-2D, which is the half-life. Additionally, it is simple to measure technically. In addition time, it is presumed that in some instances, the most appropriate and trustworthy markers for evaluating status may be Turns out to be other markers, particularly free 25-(OH)- D. This is because free 25-(OH)D is the most accurate of all the vitamin D markers (Chun *et al.* 2014).

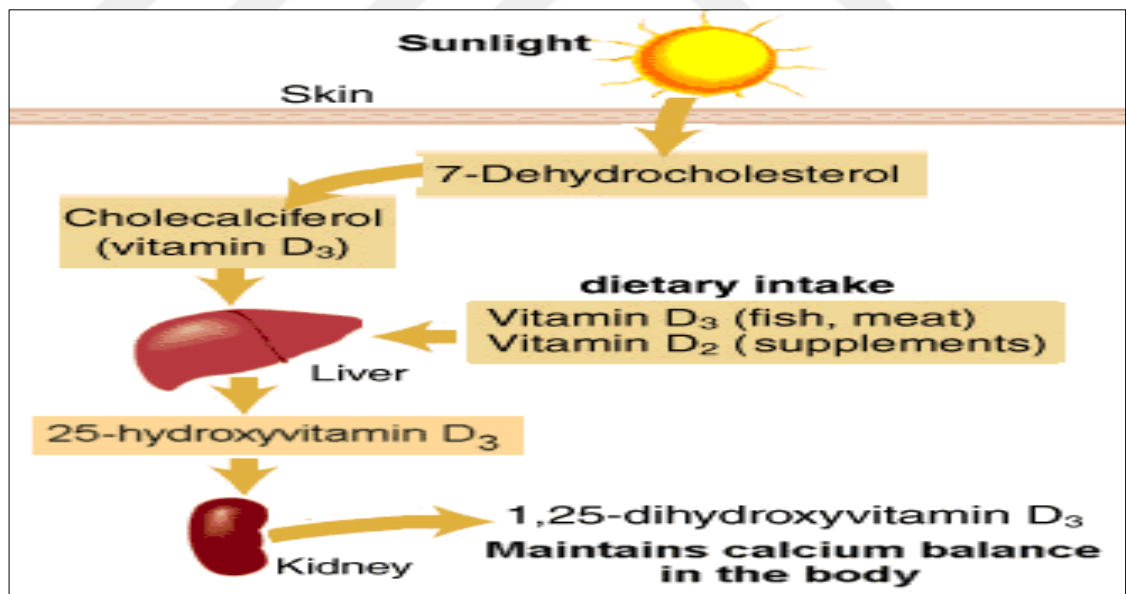


Figure 2.1 An outline of the several steps involved in the manufacture of the active vitamin

The production of vitamin D₃ begins in the skin, where 7-dehydrocholesterol is transformed into it in reaction to the action of ultraviolet B radiation. Vitamin D₃, which

may be absorbed either by the skin or through the intestinal absorption of various components of food, is hydroxylated in the liver by 25-hydroxylases. After this, the 25-hydroxyvitamin D₃ that has been produced is hydroxylated in the kidney by the enzyme 1 α -hydroxylase to produce the final active product, which is 1, 25-dihydroxy vitamin D₃ (Verstuyf *et al.* 2010).

2.3.3 Vitamin D deficiency

Fat-soluble vitamin D is crucial for maintaining calcium homeostasis and bone metabolism. Insufficient vitamin D can cause osteomalacia, rickets, and osteomalacia in adults as well as in children. In the 1930s, vitamin D fortification of milk helped to completely eradicate rickets from the planet. However 1 billion people worldwide still suffer from subclinical vitamin D deficiency affecting both industrialized and developing countries (Nair and Masih 2012).

The insufficiency is now linked to cancer, cardiovascular disease, diabetes, autoimmune illness, and depression, according to several contradictory recent research (Holick 2005). Among the elderly, low oral vitamin D consumption is more common. Between 50% and 90% of vitamin D is absorbed via the skin through exposure to sunshine, with the remainder coming from food. Vitamin D insufficiency must be avoided by getting at least 20% of the skin exposed to sunshine each day for 20 minutes (Naeem 2010).

As they age their bodies produce less vitamin D. People with darker skin have lower levels of vitamin D production. Vitamin D deficiency can be caused by reduced sun exposure and is common in hospitalized or discharged patients (Thomas 1998).

When sunscreen is continuously worn, effective UV exposure reduces. In calcium homeostasis and bone metabolism, vitamin D is crucial. In cases of chronic and/or acute vitamin D insufficiency, the intestine's decreased capacity to absorb calcium and phosphorus results in hypocalcemia, which in turn causes secondary hyperparathyroidism. The subsequent secondary hyperparathyroidism causes

phosphaturia and bone demineralization to occur quickly. Due to this, adults and children alike may develop osteoporosis and rickets as well as osteomalacia (Shen and Ji 2016).

2.3.4 Vitamin D status and DM1 risk

According to the findings of three separate meta-analyses, people with type 1 diabetes have significantly lower levels of 25(OH)D than control subjects do (Liu *et al.* 2015, Shen and Ji 2016). This lends substantial credence to the hypothesis that a shortage in vitamin D is associated with type 1 diabetes. There is a contradiction between having a vitamin D deficit and having a higher risk of developing type 1 diabetes at this time; it is not apparent whether or not the two are related. It was investigated if low levels or decrease of vitamin D cause type 1 diabetes or whether the condition develops as a consequence of another illness. Variation is common in search result patterns. Depending on the stage of life that the individuals in the study were in. We can use vitamin D supplements. In contrast to early exposure to it, for which compelling evidence has not yet been found, it has a clear favorable impact on the risk of developing DM1 at an early age. This effect is obvious and positive. Findings from two meta-analyses of observational studies support the idea that early vitamin D supplementation is associated with a lower incidence of type 2 diabetes (Levine and Gosnell 2003, Zipitis and Akobeng 2008).

It is important to highlight that the studies that were included were quite different from one another; for example, the timing of when the medicine was first administered was not taken into account. Children who took vitamin D supplementation from the seventh month to the twelfth month of life had a significantly lower risk of developing type 1 diabetes compared to children who received the supplements during the first six months of life. This finding enables us to assume that children's acquired immunity plays a role in these processes (Stene and Norwe 2003).

A large Finnish cohort study involving more than 10000 children showed that the protective effect was dose related. A 2001 study by Hypponen and colleagues found that the optimal daily intake of vitamin D is 2000 IU or more (IU).

Throughout a six-year follow-up period that involved older children, there was no correlation seen between lower levels of vitamin D and the development of type 1 diabetes (Raab *et al.* 2014). But among younger generations, People who developed DM1 throughout the year had significantly reduced levels of 25(OH)D (Gorham *et al.* 2014).

Because of this, the data that were provided demonstrate that having a suboptimal vitamin D level might be regarded as a risk factor for the formation of DM1, particularly during certain phases of life, such as in infancy. On the other hand, there is evidence that this is the case. The physiological and behavioral abnormalities that vitamin D insufficiency produces in people with type 1 diabetes may be responsible for the widespread issue of vitamin D deficiency among these patients (Simpson *et al.* 2011).

2.3.5 Vitamin D metabolism in DM1

The first attempts to identify disturbances in vitamin D metabolism in T1DM were made in early experimental studies when insulin was postulated to play an important (we can very) role in regulation. 1α -hydroxylase activity and level maintenance Serum $1,25(\text{OH})_2\text{D}$ in response to physiological stimulation of PTH. Showed that in mice with streptozotocin-induced diabetes, there is no (change) increase in activity 1α -hydroxylase and $1,25(\text{OH})_2\text{D}$ in response to calcium deprivation. This response is fully restored when insulin is administered (Ikeda *et al.* 1987).

The type 1 diabetes, who receiving insulin therapy in different regimens, An assumption has been made about a possible stimulus, the effect of insulin on 25 hydroxylases, so as in individuals who have received insulin therapy by intra peritoneal insulin infusion and have a more high concentration of insulin in the portal vein, higher levels of 25(OH)D were observed, but identical levels of $1,25(\text{OH})_2\text{D}$ compared to who got it Subcutaneous injection of insulin (Colette *et al.* 1989).

Because of decreased insulin secretion, which is a central link in the pathogenesis of DM1 disease, there may be a decreased activity of enzymes involved in vitamin D metabolism.

In recent days, publication of the work of insulin treatment options is looking very much for a longer follow-up period (more than 4 years versus several months) (van Dijk *et al.* 2019), the authors made a hypothesis about the adaptation of 5-hydroxylase activity that occurred and, as a consequence, the normalization of the Vit.D level in subcutaneously treated individuals. Additionally, the authors note that the study did not determine 1, 25(OH)₂, levels may help explain the reasons for the difference in results (Levin *et al.* 2007).

Another important cause of low 25(OH)D levels vitamin D binding protein in type 1 diabetes can cause loss of 25(OH)D in the presence of albuminuria. This is supported by a large cross-sectional analysis (Diaz *et al.* 2009). Urinary vitamin D-related protein deficiency in patients with type 1 diabetes is greater than in controls (Thraillkill *et al.* 2011, Kurosaki *et al.* 2018, Anderson *et al.* 2010).

2.4 Vitamin D Deficiency and Development Risk

2.4.1 Type 2 diabetes

Recent research has found a link between vitamin D deficiency and the risk of developing diabetes mellitus and its long-term outcome (Sung *et al.* 2012). For example, information from the Framingham Study; A 40% boom withinside the risk, of growing kind 2 diabetes in people has been shown. With at the least 25(OH) D in serum after 7 years from the begin of observation (Liu *et al.* 2010, Alam *et al.* 2016).

However it is not fully understood how vitamin D is involved in the process of glucose metabolism. However it has been proposed that vitamin D and calcium play a role in regulating glucose homeostasis and that altered levels of these nutrients may play an important role in the pathogenesis of diabetes (Quintero-Fabián *et al.* 2022). It is hypothesized that the impact of vitamin D on pancreatic islets may be mediated by the interaction of vitamin D with certain receptors and regulators. About the manifestation of several elements or a direct cause, its secretion (Lee *et al.* 1994).

Additionally, healthy intracellular levels of ionized calcium (Ca^{2+}) shield beta cells against cytokine-mediated necrosis and apoptosis as well as the production of free radicals (Rabinovitch *et al.* 2010). So thus, it alters the activity of vitamin D receptors or changes in a concentration outside and inside cells. Calcium in cases of vitamin D deficiency can harm function. The state of pancreatic cells leads to a decrease in the synthesis and secretion of insulin (Quintero-Fabián *et al.* 2022).

In addition, data indicating a possible role of vitamin D deficiency in the development of insulin resistance (Sung *et al.* 2012, Song *et al.* 2013, Bachali *et al.* 2013). Established, Vitamin D stimulates receptor gene expression. Thus, insulin is involved in the transmembrane transport of glucose (Maestro *et al.* 2000). Also found normal vitamin D levels maintain Ca^{2+} concentration inside cells tightly scale, it has a different degree of vitamin D deficiency negatively affects the activity of intracellular glucose transporter 4 (GLUT-4) and leads to the development of insulin resistance in peripheral tissues (Quintero-Fabián *et al.* 2022, Begum *et al.* 1993).

2.4.2 Rickets

Rickets is a condition of the skeleton that may be brought on by a deficiency in calcium, phosphate, or vitamin D. These nutrients are very necessary for the formation of bones that are robust and healthy. Symptoms of rickets might include skeletal deformities, weak and fragile bones, and stunted growth if the disease is allowed to progress to a severe stage (Sahay and Sahay 2013).

Vitamin D helps human body more effectively absorb calcium and phosphate from your intestines. Vitamin D is included in several foods, including milk, eggs, and fish. When you are exposed to sunlight, your body also produces the vitamin. When you don't get enough vitamin D, your body struggles to keep levels of calcium and phosphate at healthy levels. When this occurs, your body produces hormones that cause your bones to release calcium and phosphate. If you don't consume enough of these minerals, your bones will deteriorate and soften. Most rickets cases affect youngsters between the ages of 6 and 36 months. Children are more at risk for rickets due to their continued growing. Children

who don't receive enough sunshine, eat vegetarian food, or don't drink milk products may not get enough vitamin D. The condition might run in certain families (Song *et al.* 2013).

2.4.3 Vitamin D and osteoporosis

The most prevalent metabolic bone disease worldwide is osteoporosis. A known risk factor for osteoporosis is a low vitamin D level. The active uptake of calcium via cells will be reduced by insufficient vitamin D levels in the blood. Even though combining supplements of calcium and vitamin D is linked to increased bone mineral density and a decreased risk of hip fractures (Rodriguez-Martinez and Garcia-Cohen 2002).

Less apparent is the data supporting vitamin D supplementation alone. According to a recent review of the research, supplementing with vitamin D at dosages higher than 700 IU per day (with calcium) reduced bone loss more than a placebo did. However, fractures were unaffected by vitamin D administration (300–400 IU daily) without calcium. A Cochrane study concluded that calcium was also required to make a discernible impact on fracture rates and found conflicting evidence that vitamin D alone affected hip, spine, or other fracture rates (Cranney *et al.* 2008).

2.5 Calcium

Calcium is the 6th maximum customary detail withinside the human frame, and extra than 99% of it's miles contained withinside the skeletons as the complicated calcium and phosphate molecule hydroxyapatite. This mineral now no longer only offers the bones with the firmness they want to facilitate motion, however it additionally serves as a reservoir to hold steady degrees of serum calcium. Bones can not pass with out this mineral. Calcium performs a full-size function in a extensive form of capabilities which can be critical to the frame. The metabolism of this substance is managed via way of means of 3 number one delivery mechanisms: intestinal absorption, renal reabsorption, and bone turnover. Controlling the motion of calcium all through the frame is the activity of an advanced homeostatic hormonalcommunity it is made of hormones like a

parathyroid hormone (Peacock 2010). Deficits The diet is the only calcium source. Osteoporotic fracture and reduced bone mass are symptoms of calcium deficiency. Lack of calcium leads to rickets in children who are developing quickly. Kidney stones and colon cancer risk have both been linked to low levels of intestinal calcium brought on by poor dietary intake (Me 2006). This may be due to increased bile acids and decreased absorption of oxalic acid a known carcinogen and a major component of kidney stones.

2.5.1 Natural food sources of calcium

Dairy products are the main natural calcium food sources. 300 mg of calcium is included in one cup of milk or yogurt. Consumers have access to a wide variety of foods that are fortified with calcium. Soymilk supplemented with calcium has a nutritional profile that is most similar to that of dairy. Calcium-rich orange juice contains many of the nutrients that milk contains. Achieving the purity requirements without 3 servings of dairy per day is challenging because milk contains high levels of calcium phosphorus riboflavin and vitamin B-12 Committee 2010).

2.5.2 Clinical use of calcium

Typically, calcium is usually given as a dietary supplement when used therapeutically. Although the most common types are tablets many liquids are fortified with calcium which are readily available and generally easy to consume. Calcium supplements are used for three types of diseases. The first are disorders that use supplements to increase the proliferative component of absorption and eliminate malabsorption of calcium. The supplement is most effective when taken frequently and between meals (mainly given as grams of calcium). Diseases include osteoporosis malabsorptive bowel disease and hypoparathyroidism. Calcium supplementation (2 g/d) is given to treat hypocalcemia in hypoparathyroidism. However, Because hypocalcemia is caused by impaired renal calcium reabsorption dietary supplements may help increase fasting serum calcium. Dietary phosphate chelation is effective in reducing hyperphosphatemia and increasing fasting serum calcium. Calcium supplements are used to increase calcium absorption in

malabsorption bowel disorders that can occur in small bowel obstruction celiac disease and other conditions (Peacock *et al.* 2000).

Another category uses calcium supplements to treat certain dietary ion especially phosphate disorders. The goal of treatment is to reduce serum phosphate by reducing phosphate absorption and this application is mostly limited to severe renal insufficiency (Kazama 2009).

Calcium supplements work well as phosphate binders in patients with (CKD) chronic renal failure. Unlike supplements that are used to improve calcium absorption they are given at 0.5 grams per meal (Bolland *et al.* 2008) and chronic renal failure (Chertow *et al.* 2002). The third category includes conditions that cause symptoms of stomach acidity.

2.5.3 Toxicity

The daily upper limit of calcium has been established as 2500-3000 mg for adolescents 2500 mg for adults and 1000-1550 mg for infants. This is the amount of calcium the body can tolerate (Rosen and Taylor 2013). Intake above this level increases the risk of hypercalcemia and hypercalciuria kidney stone formation vascular calcification and soft tissue interactions with zinc and iron absorption and side effects such as constipation. Use an amount that is about two to three times the recommended daily intake. Continue fasting Calcium supplementation does not cause hypercalcemia if the calcium/PTH/vitamin D1/25 axis is functioning normally renal function is good and acid-base balance is adequate. However hypercalcemia can occur when this mechanism does not work properly and too much calcium is taken from food or supplements. In addition, it occurs when high calcium consumption is paired with a high alkali intake, which alters renal calcium reabsorption. One example of this is the milk-alkali syndrome (Felsenfeld and Levine 2006).

A condition known as hypercalciuria develops when the kidneys excrete more calcium than the body requires for its reserves, the majority of which is stored in bone. This excess

calcium leads to an increase in the amount of calcium that is excreted in the urine. Hypercalciuria is one of the risk factors for the formation of renal stones. This is because hypercalciuria leads to the precipitation of calcium oxalate and calcium phosphate in the urine. Conversely an increase in urinary pH and oxalate levels is a significantly greater risk factor for the formation of calcium oxalate and calcium phosphate stones respectively. These types of stones are caused by calcium oxalate (Robertson and Peacock 1980).

Mineralization is a complex process that occurs in blood vessels as well as in soft tissues and bones (Murshed and McKee 2010). The supply of calcium especially phosphate is essential but the relationship between serum calcium and reactive phosphate production and the presence of mineralization is not at all clear although mineralization is an essential process. Soft tissue mineralization is largely not directly affected by dietary calcium intake. Due to the combination of important micronutrients such as iron and zinc constipation in individuals at risk of these deficiencies is considered a side effect of high dietary calcium supplements but only a small number of individuals experience this side effect. Its pathophysiology is unknown (Murshed and McKee 2010).

2.5.4 Calcium homeostasis in the body

Calcium is necessary for the proper functioning of a lot of the body's processes, including cell growth and division, hematopoiesis, cardiovascular homeostasis, hormone responses, neuronal electrical activity, and the production of bone (Wongdee and Charoenphandhu 2017, Favus and Goltzman 2013).

This is due to the fact that a significant change in plasma calcium (fibroblast growth factor). These calcium-regulating hormones are responsible for regulating not only the amount of calcium that is found in the plasma but also the metabolism of calcium throughout the body. This ensures that calcium is absorbed in the intestine, properly stored in the bones, and that any excess calcium is excreted through the kidneys. In order to maintain a healthy calcium balance, it is necessary to take into consideration two different aspects of calcium metabolism. The first consideration is the overall quantity of

calcium found in the body, which in turn is determined by the amount of calcium that is absorbed by the intestines and the amount that is excreted by the kidneys. Individuals who lead normal lives and ingest 1000 mg of calcium per day absorb 200 mg (20%) of that amount via their intestines, and they also excrete 800 mg in their feces and 200 mg in their urine, respectively (Wongdee and Charoenphandhu 2017).

Therefore, the amount of calcium that enters and leaves the body remains constant. The maintenance of plasma ionized (Ca^{++}) calcium concentrations within the normal range of (4.65–5.2 mg/dL) is the second component. When hypocalcemia is present, there is a particular increase in the blood levels of both PTH and 1,25(OH) $_2$ D $_3$. These effects are due to PTH's role in the human body regulation of the calcium-phosphate balance in the humans body. As a consequence of the increased bone resorption brought on by HGH, calcium will be liberated into the microcirculation (Wongdee and Charoenphandhu 2017).

When hypercalcemia develops, however, the normal amount of plasma ionized (Ca^{++}) calcium is recovered as a result of negative (-) feedback processes that restrict calcium entry and promote calcium excretion. This is because hypercalcemia causes calcium entry to be restricted and calcium excretion to be promoted (Wongdee and Charoenphandhu 2017). As a part of the feedback process, the calcium-sensing receptors (CaSR) of the primary parathyroid cells detect a large quantity of ionized calcium. This suppresses the release of PTH and the creation of 1,25(OH) $_2$ D $_3$, which is a hormone that regulates calcium levels in the body (Wongdee and Charoenphandhu 2017).

Because of its ability to inhibit the intestinal calcium absorption caused by 1, 25-(OH)- $_2$ D $_3$, FGF2-2 was only recently added or/to the list of hormones that are (role) responsible for regulating calcium-absorption (Khuituan *et al.* 2012, Khuituan *et al.* 2013). Therefore, decreasing the creation of 1, 25(OH) $_2$ D $_3$ directly reduces intestinal calcium absorption, but increasing FGF23 production by osteoclasts in bone or local production in the gut indirectly inhibits intestinal calcium absorption (Wongdee and Charoenphandhu 2017, Khuituan *et al.* 2012, Wongdee *et al.* 2016).

In the 2000s, new research led to the discovery of both the role of calcium homeostasis in diabetes and the links between calcium-regulating hormones and the development of diabetes mellitus (DM). For instance, those who had low levels of vitamin D had a significantly increased factor risk of developing insulin resistance, suppurative urine syndrome, and type 2 diabetes (Heath *et al.* 1979, Yang *et al.* 2018, Chiu *et al.* 2004).

Recently, it was shown that having high levels of hCG was linked to diabetes, with different ethnic groups having greater incidences of the disease (Reis *et al.* 2016). The function of calcium-regulating organs is also impacted by DM, which results in reduced intestinal calcium absorption, deteriorated bones, slower bone elongation, and renal calcium squandering (Figure 2.2). More information is provided below.

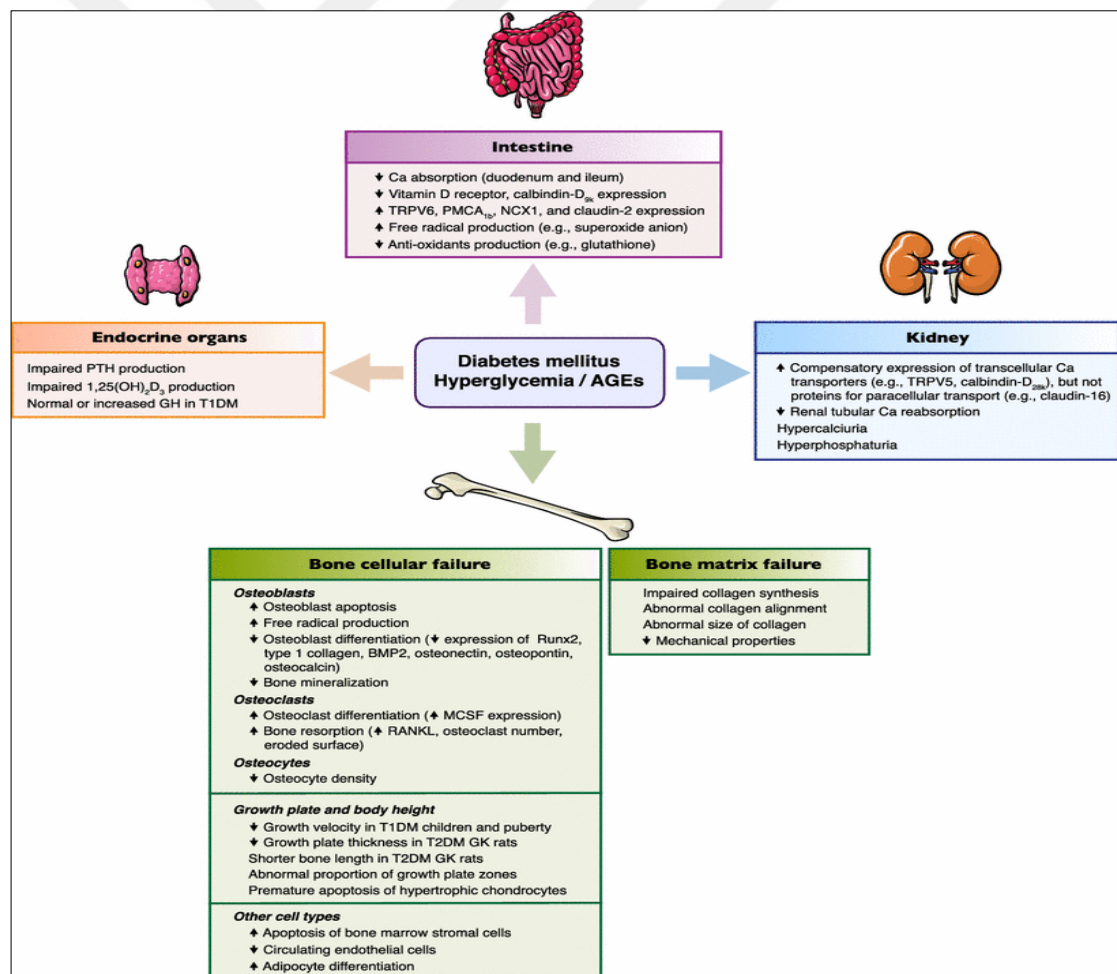


Figure 2.2 The metabolism influence of calcium-regulating chemicals.

3. MATERIALS AND METHODS

3.1 Materials

3.1.1 Deviced and tools

Table 3.1 shows tools and some other devices used in the current investigation.

Table 3.1 Some important materials, and tools during the current study

Devices and tools	Origins
Frozen Centrifuge	USA
Water bath	Germany
Incubator	Korea
VIDAS analyzer	Bio Merieux clinical diagnostics, England
Pipettes for separating serum	Germany
Spectrophotometer	Apple, USA
CobAS C111analyzer	Atlas Bion Corporation, England
Analyzer	Beijing DiaGreat Biotechnologies co., Ltd
Micropipete 10-100-1000 μ L	China
Centrifuge	Wise Clave, Korea

3.1.2 Study population

In this study, 150 person studied the estimate of the effect of vitamin D levels and calcium levels in children with diabetes disease. The groups of this study were classified into two groups as in Figure 3.1.

- 1- Group A includes 60 healthy individuals (Control group).
- 2- Group B includes 90 Diabetes Mellitus patients (patient group).

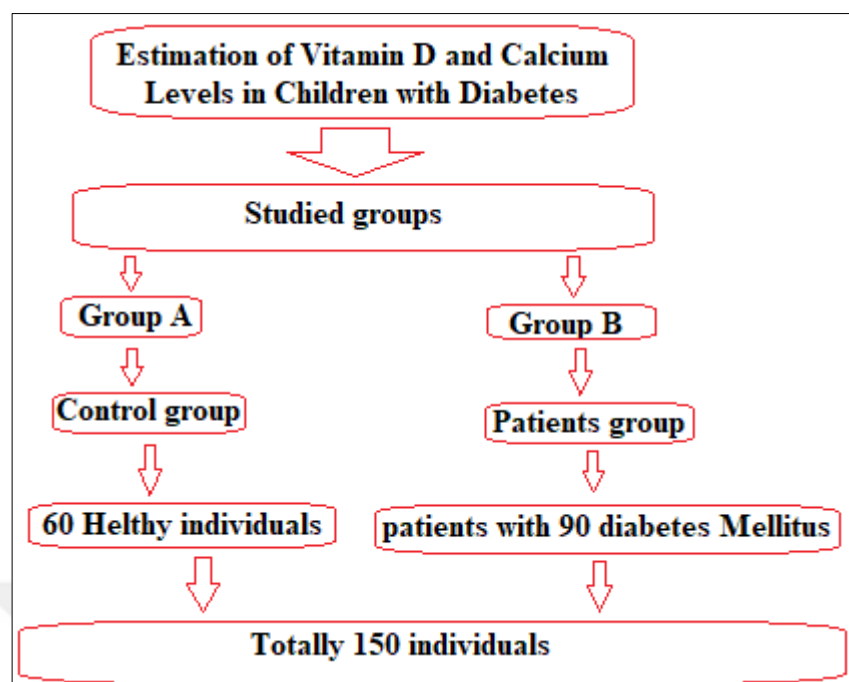


Figure 3.1 Studied groups

3.1.3 Blood samples

It was obtained the blood samples of this study from the patients, 5 mL of blood was collected by using sterile medical syringes from the brachium vein, and I placed in plain tubes, then left in the tube at 25°C for 30 minutes to coagulate the blood and then centrifuge the blood samples at 2500 rpm/min for 5 minutes to separate the serum from other ingredients of blood, the serum will be segregated and tows the serum by micropipette then put it in other tubes for conduct biochemical tests.

3.2 Methods

3.2.1 Vitamin D test

The blood sample was obtained in a serum separator tube, and we waited for it to clot before proceeding. The samples are going to be centrifuged for ten minutes at a range of 1100 to 2000 g. Immediately separate the serum from the cells, and then store the serum at 4 degrees Celsius until further processing. Samples that are severely haemolysed,

lipemic, or contain particle material should be discarded. After that, I used an appropriate immunoassay on the Cobas C111Analyzer to determine the amounts of vitamin D (Bion Corporation, England) then induct the test curve card into the analyzer and read the card, induct the information of the samples including sample number, type, and test item, click start the sample treatment, then I was added 20 μL of sample to the buffer A, use a new tipper and added 30 μL buffer B to the previous mixture. Mix for 5 seconds then place the sample in the incubator for 15 minutes, then I added 80 μL of sample to the cassette. Induct the cassette and start incubation for 10 minutes then I inducted the cassette into the analyzer and I was push the “quick test“ the machine will test and print the results.

3.2.2 HbA1c test

The HbA1c procedure measured the average blood glucose levels and I took the blood samples and I was analyzing the results. Induct the cartridge into the machine at 30°C then I added 100 μL of hemolysis buffer into the buffer tube. Take away 5 μL of blood then induct the blood into the buffer tube and shake it 15 times. Add 75 μL of sample mixture in the cartridge sample and then march out the cartridge from the machine and arrange the mixture onto it, incubate at 30°C. Verify that the mixture flow well on the membrane of the cartridge (+/- 10 seconds), the sample was embosomed for 12 minutes at 30°C, then insert the cartridge and run the test and finally the results will be displayed on the screen after the running will be finished.

3.2.3 Complete blood count

A complete blood count will be checked to count the red blood cell (RBC) levels. Blood samples will be analyzed within one hour.

3.2.4 Calcium test

I brought three test tubes (blank, standard, and specimen) and I added to each tube 0.5 mL of working reagent R1 and 0.5 mL of working reagent R2 then add 25 μ L of distilled water to the blank tube, 25 μ L of standard solution to standard tube, and 25 μ L of the blood sample to sample tube then Incubate the tubes for 5 minutes at 25°C, read the absorbance at 500 nm and record the results, Equation (3.1) was used to calculation the concentration of calcium.

$$\text{Calcium (mg / dL)} = (A_{\text{specimen}} / A_{\text{standard}}) \times \text{Concentration (Standard)} \quad (3.1)$$

3.2.5 PTH test

I Carried the reagents and put the cartilage into the selected position then placed the code tube in position #1 of the ring then I pressed the scan key to allow the machine to scan all the reagents, and after that, I checked the calibration key by loading the calibrators and controls then I have opened the covers and then I cleaned the cell, reagents, calibrators, and controls. After the cleaning I pushed the start key and then pushed the calibration key, next the calibration is finished the results will disappear, I was loading the sample onto the sample reagent deck, and afterward, I pressed the scan key button to register the samples next I pressed close key then pressed orders and sample orders next select PTH test for each sample. The machine will calculate all the results automatically.

3.2.6 Urea test

The principle of the urea test is include the hydrolyzation of urea by assisting urease to form ammonia and CO₂. then ammonia reacts with salicylate and sodium nitroprusside with the formation of the chromophore, the formed color intensity is proportional to the urea concentration in the sample.

The reagents composition of this test is consist of R1 reagent (urease > 500 U/mL), R2 reagent (buffered chromogen, phosphate buffer 20 mmol/ L, EDTA-2 mmol/L, and sodium salicylate(60 mmol/L), R3 reagent (alkaline hypochlorite), CAL (urea 50 mg/L).

Procedure: I brought the reagents and samples at 25°C then pipette into the cuvette 1 mL from each of the blank, sample, and CAL standard into the reagent tube, 10 µL of sample into the sample tube, 10 µL of cal standard into cal standard tube, then I have added 1 mL from each of blank, sample and cal standard into R3 reagent tube, mix and incubate the tubes for 5 minutes at 37°C then read the absorbance at 600 nm of samples and standard against the blank. Equation (3.2) was used to calculation urea concentration.

$$\text{Urea concentration (mg/dL)} = (A_{\text{sample}} / A_{\text{standard}}) \times C_{\text{standard}} \quad (3.2)$$

3.2.7 Creatinine test

The principle is based on the reaction of creatinine with picrite ions under alkaline conditions with the formation of a complex. The absorbance was measured through the formation of a complex that is proportional (most be careful) to the creatinine concentration (level) in the sample. Equation (3.3) used creatinine reagent.



The reagents composition: Consist of R1 reagent (Picric acid 25 mmol/L), R2 reagent (Alkaline buffer/Phosphate buffer 300 mmol/L, SDS 2 g/L), and CAL (Creatinine standard/creatinine 2 mg/dL, organic matrix based primary standard).

Procedure: At first, I set the spectrophotometer to zero absorbance, then I pipette into the cuvette 1 mL from the reagent with 100 µL of the sample then mix and induct the cuvette then I read the absorbance at 510 nm after 30 seconds (A1), then after 90 seconds (A2) of the sample addition. Equation (3.4) was used to calculations creatinine concentration.

$$\text{Creatinine (mg/dL)} = \{(A2-A1)_{\text{Sample}} / (A2-A1)_{\text{Standard}}\} \times C_{\text{Standard}} \quad (3.4)$$

3.2.8 Alkaline phosphatase test

Preparation of the working reagents: I was prepare ALP buffer (R1 reagent) by mixing 1.25 mol/L of DEA buffer pH 10.2 with 0.6 mmol/L of magnesium chloride, ALP substrate (R2 reagent) consisting of 50 mmol/L of 4-NPP.

Reagent preparation: At first, I mixed 4 mL of R1 reagent with 1 mL of R2 reagent, the photometer was set to zero nm with distilled water and I was added into a cuvette and mixed 1 mL of working reagent with 20 μ L of sample and then I have inducted the cuvette into the cell holder, then incubate for 1 minute and read the absorbance, then I was retried this step after 1, 2 and 3 minutes, and I have calculated the difference between absorbance and calculate the average change in absorbance per minute ($\Delta A / \text{min}$) as shown in Equation (3.5).

$$U / L = \Delta A / \text{min} \times 2764 \quad (3.5)$$

The sample was diluted 1: 2 with saline and assayed again, multiple the result by Equation (3.6).

$$U / L \times 0.01667 = \mu\text{Kal} / L \quad (3.6)$$

3.2.9 Ferritin test

The serum ferritin concentration was assayed by utilizing a commercial kit (Biosystems, Spain). The ferritin concentration in ng/ml was determined by Equation (3.7).

$$\text{Serum ferritin concentration (ng/mL)} = (A2-A1)_{\text{sample}} / (A2-A1)_{\text{standard}} \times C_{\text{standard}} \quad (3.7)$$

The agglutination of latex particles coated with anti-human ferritin that react with ferritin samples is the fundamental process that underpins this test's methodology. Turbidimetry is used to determine the latex particle agglutination, which is proportional to the ferritin content in the sample and may be quantified.

The composition of the reagent comprises of R1 Diluent reagent (Glycine buffer 20 mmol/ L pH 8.5), R2 Latex reagent (Latex particles coated with polyclonal anti-human ferritin antibodies, pH 8.2), and CAL Calibrator (Ref. 3940005 13 mL, Human ferritin).

Procedure: I added into a curve 0.8 mL of R1 reagent with 100 µL of the sample, calibrator or blank with 0.2 mL of R1 reagent, then I was mix and read the absorbance immediately (A1) and after 8 minutes (A2) of the reagent R2 addition. Equation (3.8) was used to calculate ferritin concentration:

$$\text{Serum ferritin concentration (ng/mL)} = (A2 - A1)_{\text{sample}} / (A2 - A1)_{\text{standard}} \times C_{\text{standard}} \quad (3.8)$$

Ferritin concentration in the sample is calculated by interpolation of its (A2 – A1) in the calibration curve.

3.2.10 Growth hormone test

The principle of the growth hormone test is based on the reaction of a biotinylated monoclonal antibody with native antigen and enzyme-labeled antibody to form a soluble sandwich complex, then the complex reacts with streptavidin to form an immobilized complex. After a few minutes, the antibody is separated from the antigen by pouring or aspiration, and the enzyme activity in the antibody is proportional to the concentration of the native antigen.

Reagents composition: Growth hormone calibrators 1 mL/ vial, high tracer reagent 13 mL/ vial, light reaction walls 96 wells microplate coated with streptavidin, wash solution concentrate 20 mL/ vial, signal reagent B 7 mL/ vial.

Procedure: Add 50 µL of serum (specimen, control, or calibrator) into the well then add 100 µL of high GH reagent to all wells, vortex the microplate for 25 seconds then incubate for 45 minutes at 25°C. Discard the microplate contents and dry the plate with absorbent paper. Add 350 µL of wash buffer and repeat this step five times then add 100 µL of working reagent to all wells then incubate the wells in dark for five minutes, and read the relative light units for 0.2 - 1 second of each well.

3.3 Statistical Analyses

SPSS 22.0 for windows will be used to conduct the statistical analysis of the results. Calculations will be done to determine the mean of each variable as well as its standard deviation (SD). The analysis of variance F test, also known as ANOVA, will be carried out to compare the outcomes of every case study within every group that was researched. The student's t-test was used to examine the significance of the differences in mean values across all of the variables that were examined. The correlation coefficient will be used to demonstrate the Pearson correlations that were found between the blood levels of vitamin D and calcium (r). When P is less than 0.05, the results will be regarded as insignificant; otherwise, they will be regarded as significant.

4. RESULTS AND DISCUSSION

In this study, 150 people will be studied for the estimate of the effect of vitamin D levels and calcium levels in children with diabetes disease. The groups of this study were classified into three groups.

4.1 The Gender and Study Groups

The results illustrated in (Table 4.1) revealed that there is a significant difference in gender parameters of the two study groups (patients group and healthy individuals control group) whereas it seems that the males were more sensitive to diabetes than females, the percentage of males with diabetes disease in patients group and control group recorded (71.11 and 73.33) % respectively as compared to percentage of females (28.88 and 26.66) % (Table 4.1). Also, Table 4.2 revealed there was a positive correlation between the gender and the tested groups which recorded $r = 0.768$.

Table 4.1 The gender parameter and study groups

Parameter		Group of study		Total
		Patient	control	
Gender	Male	64	44	108
	%	71.11%	73.33%	-
	Female	26	16	42
	%	28.88%	26.6%	-
Total		90	60	150

Table 4.2 Correlation and Sig. between gender and study groups

		Gender	Group of study
Gender	Pearson Correlation	1	-0.024
	Sig. (2-tailed)		0.768
	N	150	150
Group of study	Pearson Correlation	-0.024	1
	Sig. (2-tailed)	0.768	
	N	150	150

4.2 The Weight and Age of the child and the Study Groups

The results of (Table 4.3) showed that there is no significant in weight and age of the tested groups, the mean weight for the groups of this study ranged between 24.21 kg in the patient group and 21.15 kg in the control group, whereas the mean of age of the groups was ranged between 7.58 year in the control group to 8.40 years in the patient group.

Table 4.3 The weight and age of the child and the study groups

Group of study		Age of child (Year)	Weight (Kg)
Patient	Mean	7.5889	21.1556
	N	90	90
	Std. Deviation	2.32183	5.92066
Control	Mean	8.4000	24.2167
	N	60	60
	Std. Deviation	3.26356	6.35688
Total	Mean	7.9133	22.3800
	N	150	150
	Std. Deviation	2.75617	6.26128

4.3 Vitamin D and Diabetes Disease in Children

The results of the study the estimation of vitamin levels showed that levels of vitamin D were correlated with diabetes in children diabetes reduced the levels of vitamin D in the patient's group which recorded 15.54 ng/mL as compared to the control group which recorded 27.25 ng/mL respectively (Table 4.4). Vitamin D deficiency is associated with a decrease in realized of insulin and with the resistance of insulin in children diabetes of type 2 (Zipitis and Akobeng 2008). These findings were in agreement with the findings of Tytusa *et. al.* who found a decreased in vitamin D levels in 84.83% of boys and 96.9% of females in primary school children in Ukraine (Tytusa *et al.*2022). Vitamin D plays a critical role in the regulation of calcium suction and secretion, inadequate intake of vitamin D in children can perform in deficiency (Zitterman 2003). Savastio *et al.* concluded that children with type 1 diabetes mellitus reveal a deficiency in vitamin D that effect metabolic status and glycemic homeostasis (Savastio *et al.* 2016). Whereas, Zinitis and Akobeng revealed that the supplementation of vitamin D in early childhood may offer protection against diabetes mellitus development (Zinitis and Akobeng 2008).

Table 4.4 Vitamin D concentration and diabetes disease in children

Group of study	Mean	N	Std. Deviation
Patient	15.5422	90	2.33257
Control	27.2500	60	4.52348
Total	20.2253	150	6.66861

A study on the correlation between vitamin D and diabetes showed a positive correlation between them which recorded 0.630** (Table 4.5 and Figure 4.1) with a P value at 0.01 level. HbA1C is a wholesome predictor of the risk of diabetes in children and can be performed to characterize prediabetes in children with other risk factors of type 2 diabetes they concluded that the level of HbA1C was 5.5% in 238 children of 2095 patients (Vijayakumar *et al.* 2017). Whereas Pinhas-Hamiel *et al.* indicated that the levels of HbA1C declined to the lowest values in the third decade (Pinhas-Hamiel *et al.* 2014). These results were in agreement with the results of Liu *et al.* who found indicated that there is a significant correlation between serum vitamin D levels and diabetes type 1 in children (Liu *et al.* 2022).

Table 4.5 Correlation and Sig. between vit.D and diabetes in children

		Vitamin D conc	Group of study
Vitamin D conc	Pearson-Correlation	1	0.630**
	Sig.(P)		0.000
	No. of sample	121	120
Group of study	Pearson-Correlation	0.630**	1
	Sig.(P)	0.000	
	No. of sample	120	120

** Correlation is significant at the 0.01 level (2-tailed).

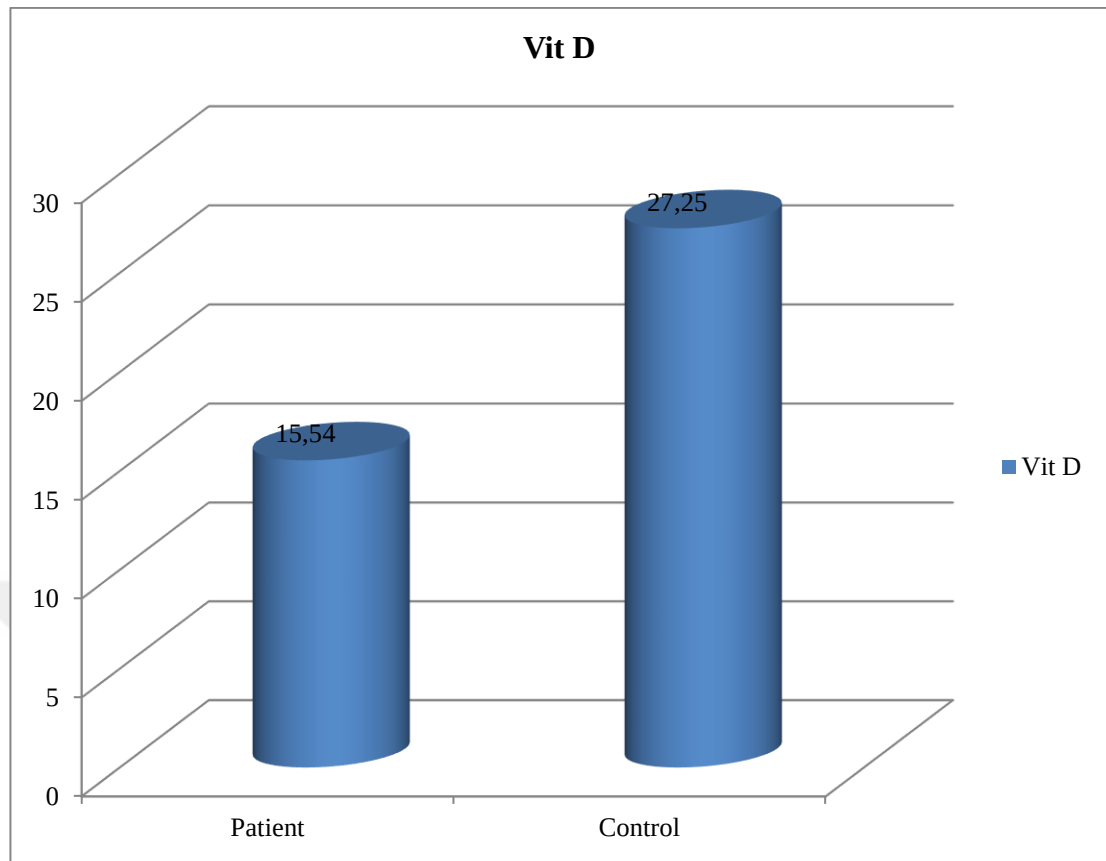


Figure 4.1 Vitamin D Levels in patient and control groups

4.4 HbA1C and Diabetes Disease in Children

The results of Table 4.6 revealed that there were no significant differences in the mean of HbA1C between the patient and control groups. The patient group recorded 6.64% as compared to 6.12% in the control group (Table 4.7 and Figure 4.2). Data showed that there is a negative significant correlation between HbA1C and diabetes disease (-0.050).

Table 4.6 HbA1C and diabetes

Group of study	Mean	N	Std. Deviation
Patient	6.6456	90	0.66942
Control	6.2100	60	6.81366
Total	6.4713	150	4.32400

Table 4.7 Correlation and Sig. between HbA1C and diabetes disease in children

		Group of study	HbA1C
Group of study	Pearson-Correlation	1	-0.050
	Sig.(P)		0.547
	No. of sample	150	150
HbA1C	Pearson-Correlation	-0.050	1
	Sig.(P)	0.547	
	No. of sample	150	150

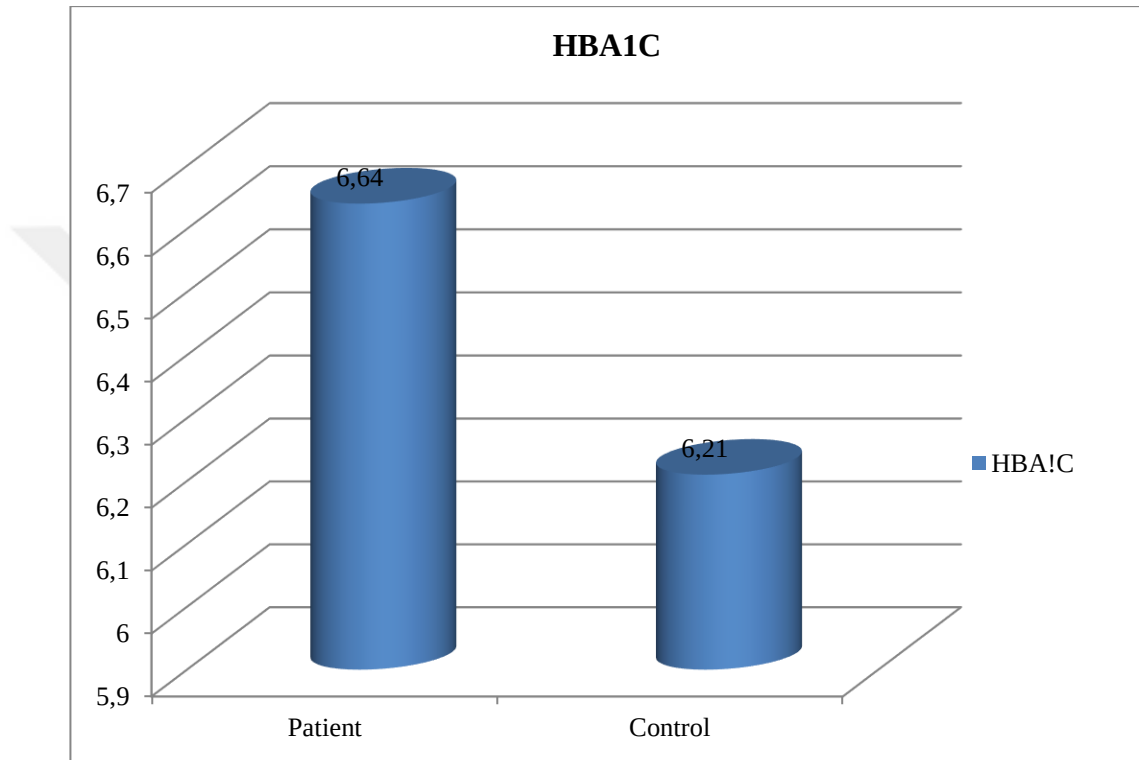


Figure 4.2 HBA1C level in study groups

4.5 Red Blood Cells (RBC) and Diabetes Disease in Children

Data illustrated in (Table 4.8) revealed that there was a significant difference in the mean of RBC between the patient group and control group, the patient group significantly elevated the RBC count which recorded 7.11 million cells/mL as compared to 4.99 million cells/ mL in healthy individuals (control) group. The elevation in RBC count may be related to high blood sugar in children with diabetes (hyperglycemia) which increased the count of RBC in a blood sample of children (Alamri *et al.* 2019).

Table 4.8 RBC and diabetes disease in children

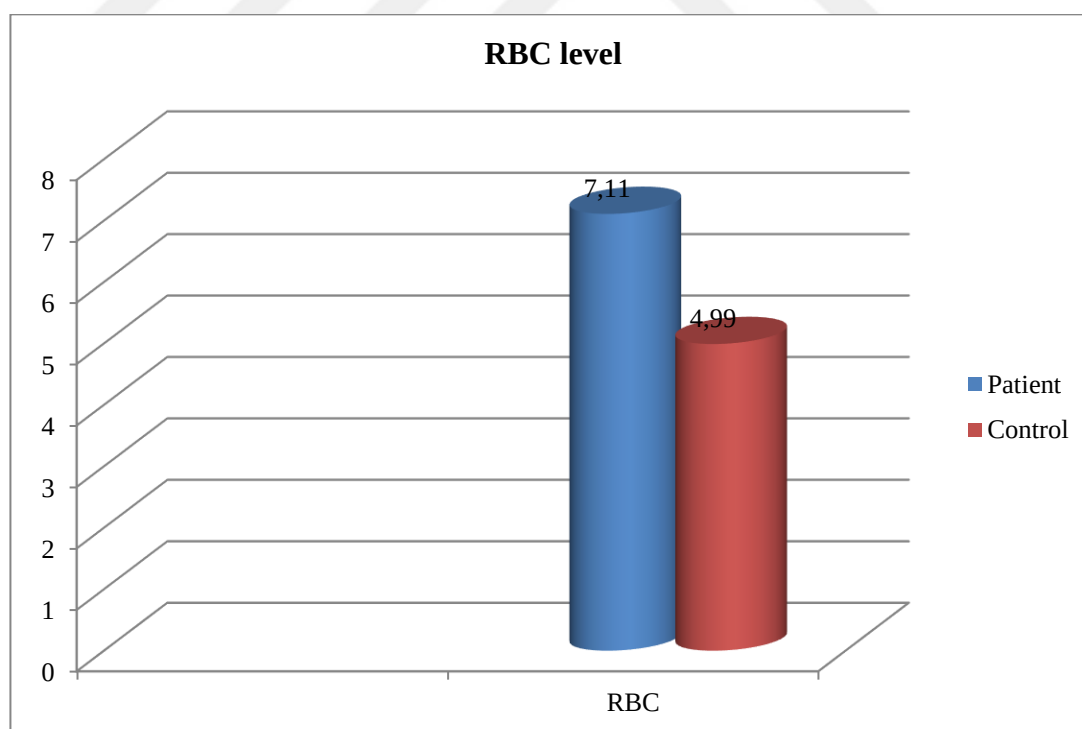
Group of study	Mean	N	Std. Deviation
Patient	7.1156	90	5.47698
Control	4.9975	60	0.45626
Total	6.2683	150	4.36854

Also, the data from (Table 4.9 and Figure 4.3) showed there was a negative correlation between RBC and diabetes (- 0.238**) with a P value of 0.01 level.

Table 4.9 Correlation and Sig. between RBC and diabetes disease in children

		Group of study	RBC
Group of study	Pearson-Correlation	1	-0.238**
	Sig.(P)		0.003
	No. of sample	150	150
RBC	Pearson-Correlation	-0.238**	1
	Sig.(P)	0.003	
	No. of sample	150	150

** Correlation is significant at the 0.01 level (2-tailed).

**Figure 4.3** RBC level and study groups

4.6 Calcium Concentration (Ca) and Diabetes Disease in Children

According to the findings in (Table 4.10), the Ca concentration of the patient group was substantially higher than that of the healthy control group. The patient group recorded 7.14 mg/dL, whereas the healthy individual's control group only recorded 5.05 mg/dL. According to research done by Becerra-Tomás and colleagues in 2014, an elevated level of serum calcium in the blood of children who have diabetes is connected with both glucose metabolism and the risk of developing diabetes.

Table 4.10 Ca concentration and diabetes disease in children

Group of study	Mean	N	Std. Deviation
Patient	7.1444	90	2.14963
Control	5.0575	60	0.27213
Total	6.3097	150	1.96004

Data from (Table 4.11 and Figure 4.4) revealed that there is a negative correlation between calcium level and diabetes in children, the correlation is significant at 0.01 level. Hanan *et al.* found that the level of serum calcium in diabetes patients was lower than in healthy individuals and the calcium level was significantly correlated with fasting blood sugar level in children with insulin-dependent diabetes mellitus (Hanan *et al.* 1999).

Table 4.11 Correlation and Sig. between Ca concentration and diabetes disease

		Group of study	Ca conc
Group of study	Pearson-Correlation	1	-0.523**
	Sig.(P)		0.000
	No. of sample	150	150
Ca conc	Pearson-Correlation	-0.523**	1
	Sig.(P)	0.000	
	No. of sample	150	150
**Significant correlation at the 0.01 level (2-tailed)			

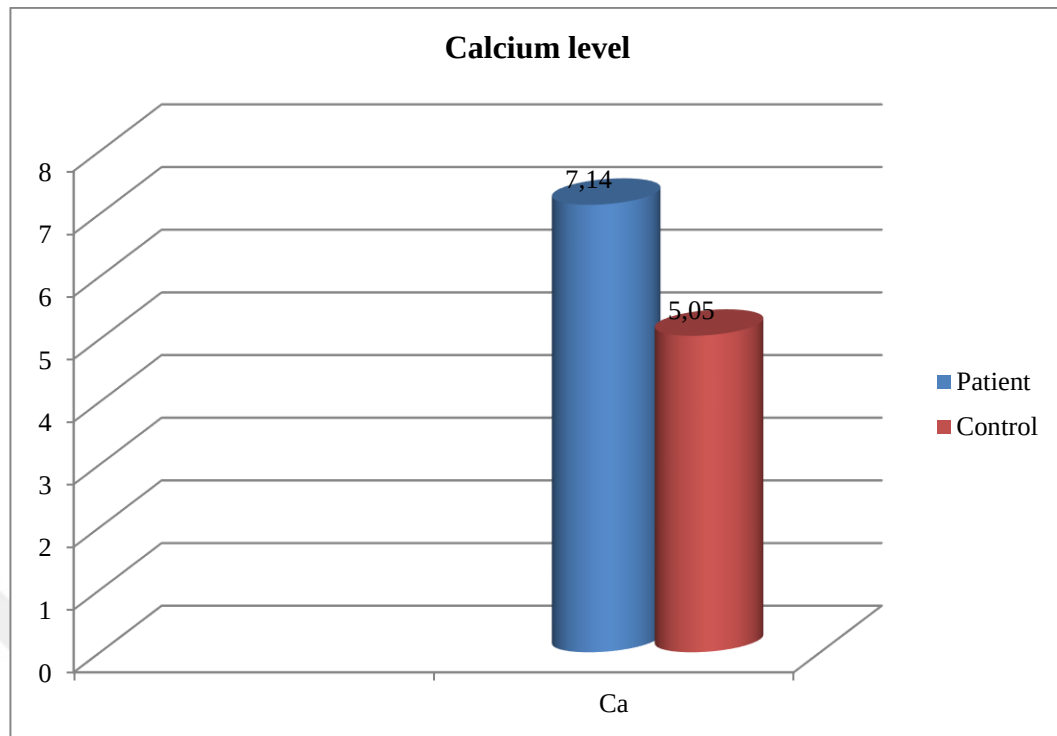


Figure 4.4 Calcium level and study groups

4.7 Parathyroid Hormone (PTH) and Diabetes

The results of (Table 4.12, Table 4.13, and Figure 4.5) indicated significant differences in the mean PTH level between patient and control groups, the patient group showed significant elevation in PTH level which recorded 80.42 pg/mL as compared to 31.95 pg/mL in the control group. The elevation in PTH level led to an increase in the calcium level which can cause a problem in the health of patients with diabetes. Also, the results showed a negative significant correlation between PTH level and diabetes (-0.912) with a P value of 0.01 level.

Table 4.12 PTH conc

Group of study	Mean	N	Std. Deviation
Patient	80.4222	90	9.97410
Control	31.9500	60	11.76957
Total	61.0333	150	26.11421

Table 4.13 Correlation of PTH and Sig. with diabetes in children

		Group of study	PTH conc
Group of study	Pearson-Correlation	1	-0.912**
	Sig.(P)		0.000
	No. of sample	150	150
PTH conc	Pearson-Correlation	-0.912**	1
	Sig.(P)	0.000	
	No. of sample	150	150

** Significant correlation at the 0.01 level (2-tailed).

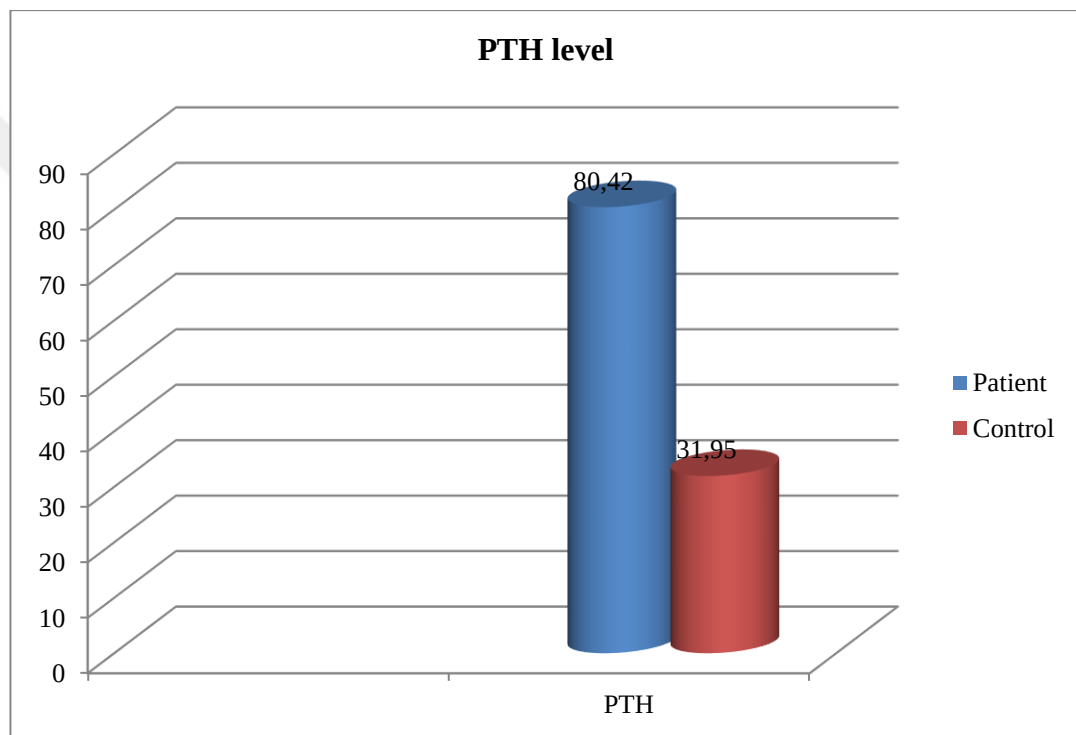


Figure 4.5 PTH level and study groups

4.8 Urea Level and Diabetes

In the study that looked at the effect of urea level on the development of diabetes in children, the researchers found that the urea level in the patient group was significantly higher than the urea level in the healthy control group (Table 4.14, Table 4.15, and Figure 4.6). The patient group recorded 57.73 mg/dL, while the control group recorded 27.11 mg/dL. The higher urea level may be related to insulin secretion from pancreatic beta cells (Xie *et al.* 2018). It's possible that people with diabetes mellitus have elevated levels

of blood urea because their kidneys aren't filtering waste products as effectively as they should be, which results in an accumulation of waste products throughout the body. According to the findings of a study which diabetic individuals with a higher blood urea nitrogen to creatinine ratio were at an increased risk of developing coronary artery disease (Liu *et al.* 2022). In addition, the findings demonstrated that there is a significant negative correlation that was observed (-0.876), and the corresponding P value was 0.001.

Table 4.14 Urea level and diabetes

Group of study	Mean	N	Std. Deviation
Patient	57.7333	90	9.05017
Control	27.1167	60	7.10238
Total	45.4867	150	17.18659

Table 4.15 Correlation and Sig. between urea level and diabetes

		Group of study	Urea level
Group of study	Pearson-Correlation	1	-0.876**
	Sig.(P)		0.000
	No. of sample	150	150
Urea level	Pearson-Correlation	-0.876**	1
	Sig.(P)	0.000	
	No. of sample	150	150

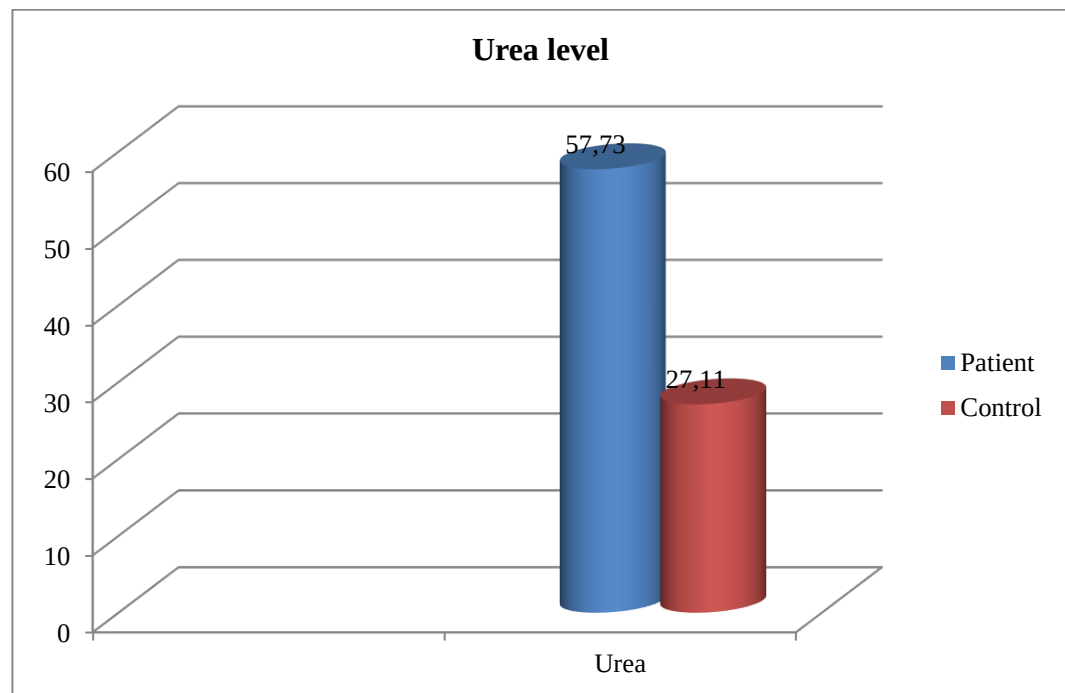


Figure 4.6 Urea level and study groups

4.9 Creatinine Level and Diabetes

The data of (Table 4.16, Table 4.17, and Figure 4.7) pointed that there is a significant difference in mean creatinine levels between the two tested groups (patient and control groups), the patient group significantly elevated the level of creatinine in blood serum as compared to healthy persons, the patient group recorded 1.55 mg/dL as compared to 0.80 mg/ dL in the control group. These findings were in agreement with the findings of a study which concluded that creatinine levels in patients with diabetes mellitus type 2 were higher than their level in the control group (Donije *et al.* 2011). Data from Table 4.17 showed that there is a negative significant correlation between the concentration of creatinine and diabetes which was recorded (-0.850).

Table 4.16 Creatinine and diabetes

Group of study	Mean	N	Std. Deviation
Patient	1.5506	89	0.22969
Control	0.8010	60	0.22934
Total	1.2487	149	0.43404

Table 4.17 Correlation and Sig. between creatinine and diabetes

		Creatinine conc	Group of study
Creatinine conc	Pearson-Correlation	1	-0.850**
	Sig.(P)		0.000
	No. of sample	149	149
Group of study	Pearson-Correlation	-0.850**	1
	Sig.(P)	0.000	
	No. of sample	149	150

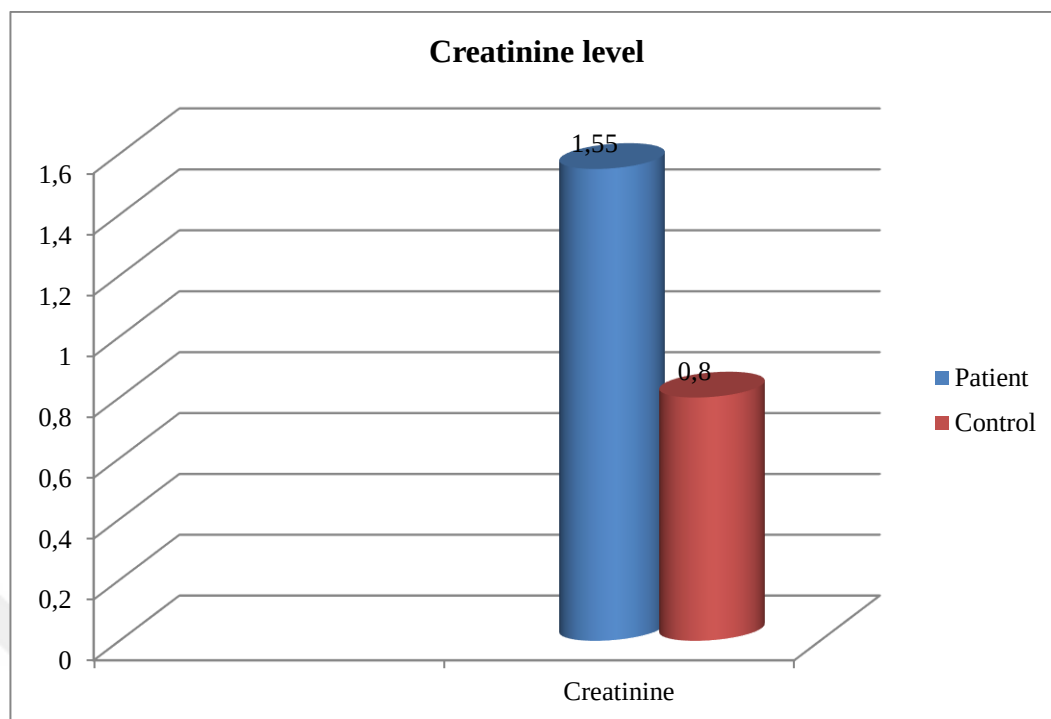


Figure 4.7 Creatinine level and study groups

4.10 Alkaline Phosphatase and Diabetes

The finding of Table 4.18 appeared that the patient group significantly excelled in alkaline phosphatase levels in the blood serum of patients with diabetes as compared to healthy persons, the patient group registered 177.25 U/L as compared to 0.92 U/L in the control group. These results were compatible with the results of Zhang *et al.* who found that levels of ALP in patients with diabetes in adults were higher than their level in healthy individuals, and indicated the increase in ALP level was significantly associated with an elevated risk of diabetes (Zhang *et al.* 2020). The alkaline phosphatase hormone considers one of the liver function checkups and the liver may be linked with diabetes (Kunutsor *et al.* 2013). The elevation of ALP level worked as a prognostic indicator in diabetes patients and possibly in connection to fallen renal function in patients with diabetes (Porter *et al.* 2016).

Data from (Table 4.19 and Figure 4.8) showed that there is a negative significant great correlation between ALP and diabetes which reported (-0.917) with the P value at 0.01 level.

Table 4.18 ALP and diabetes

Group of study	Mean	N	Std. Deviation
patient	177.2556	90	9.31299
control	92.8667	60	26.37440
Total	143.5000	150	45.25346

Table 4.19 Correlation and Sig. between ALP and diabetes

Table 4.19 Correlation between ALP and diabetes			
		Group of study	ALP conc
Group of study	Pearson-Correlation	1	-0.917**
	Sig.(P)		0.000
	No. of sample	150	150
ALP conc	Pearson-Correlation	-0.917**	1
	Sig.(P)	0.000	
	No. of sample	150	150

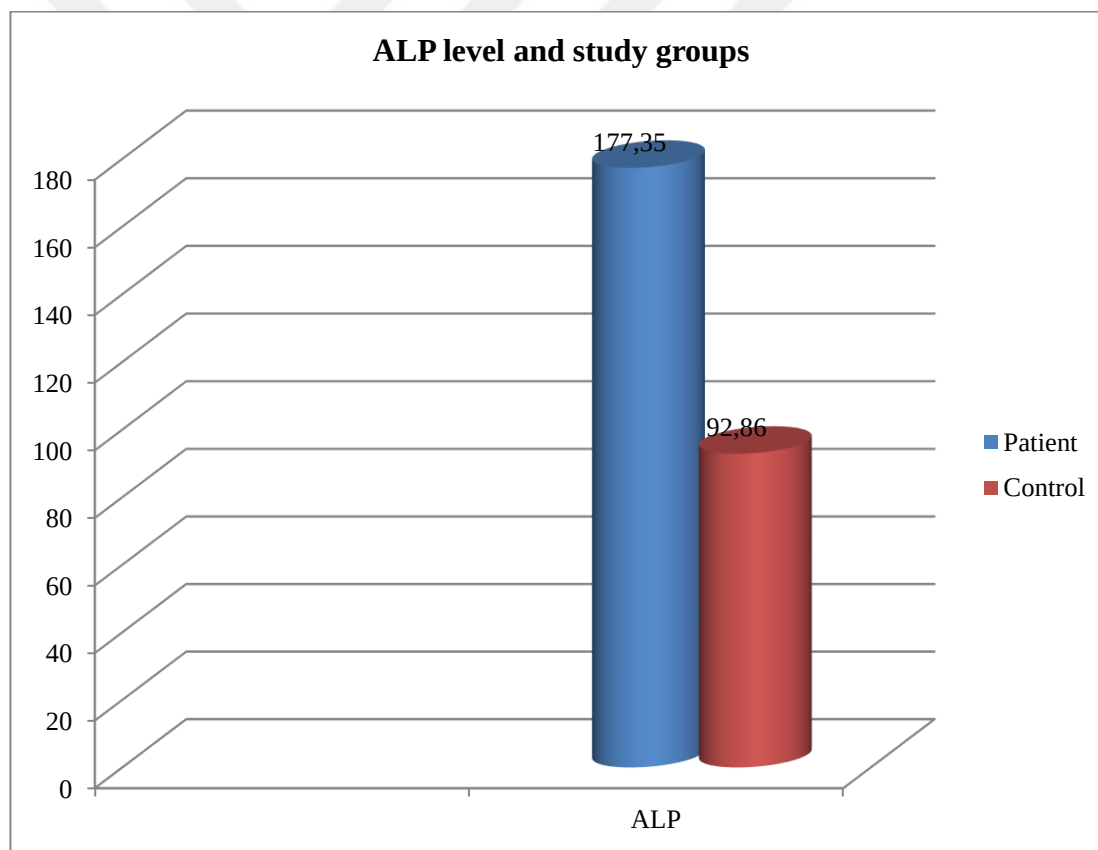


Figure 4.8 ALP level and study groups

4.11 Ferritin and Diabetes

The data of (Table 4.20) revealed that the patient group was significantly excellent in ferritin level as compared to healthy persons (control group), the patient group recorded 180.06 ng/mL as compared to 70.55 ng/mL. The increase in ferritin level may assist to circumscribes a high-risk population of diabetes patients who perhaps benefit from more assets, serum ferritin levels articulates the situation of iron reserves in healthy persons, and several studies have found increased ferritin levels linked with such diabetic intricacies as retinopathy, nephropathy, and vascular dysfunction in diabetes patients and with elevated fasting blood glucose (Daniel *et al.* 2011). These findings were compatible with the findings of (Daniel *et al.* 2011) who reported that serum ferritin levels were upper in diabetes patients type 2 as compared with healthy persons. Also, the results indicated that there is a negative significant correlation between ferritin and diabetes (-0.837**) with a P value at level 0.01 (Table 4.21 and Figure 4.9).

Table 4.20 Ferritin level and diabetes

Group of study	Mean	N	Std. Deviation
Patient	180.0667	90	32.17362
Control	70.5500	60	39.67855
Total	136.2600	150	64.33954

Table 4.21 Correlation and Sig. between ferritin and diabetes

		Group of study	Ferritin level
Group of study	Pearson-Correlation	1	-0.837**
	Sig.(P)		0.000
	No. of sample	150	150
Ferritin level	Pearson-Correlation	-0.837**	1
	Sig.(P)	0.000	
	No. of sample	150	150

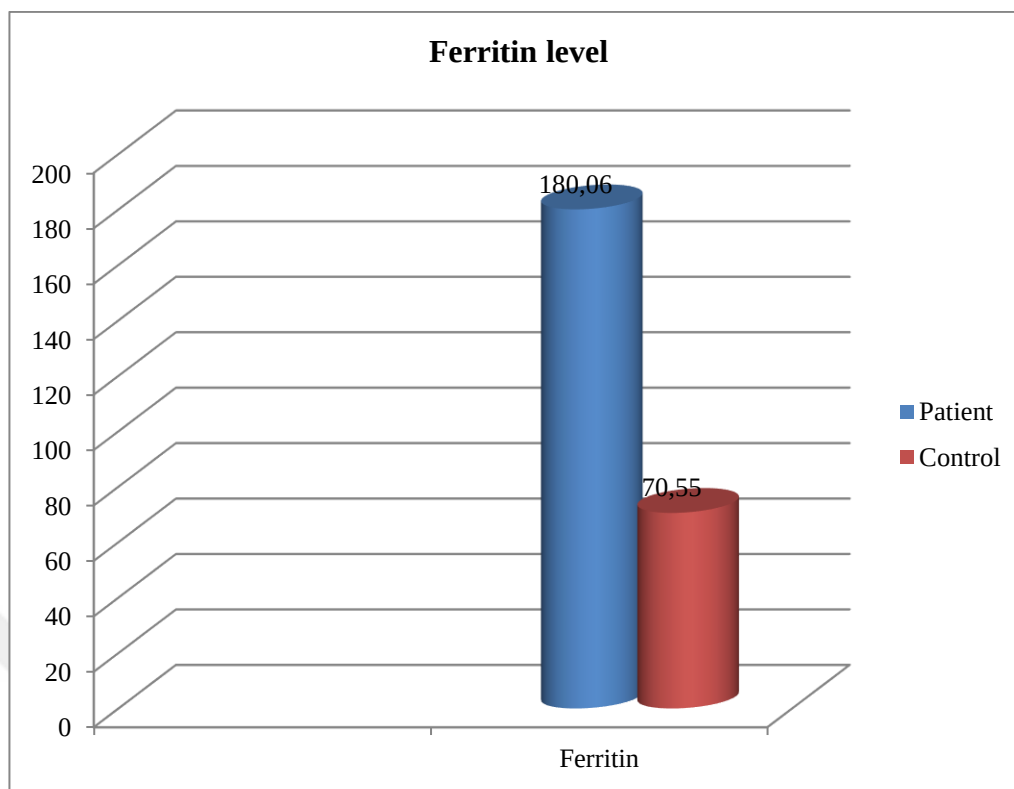


Figure 4.9 Ferritin level and study groups

4.12 Growth Hormone and Diabetes

The data of (Table 4.22) showed that there is a significant difference in growth hormone levels in both groups (patient and control) which indicated that the patient group significantly reduced the level of growth hormone as compared to healthy individuals, the patient group reported 17.71 ng/mL as compared to 33.76 ng/mL. Growth hormone is considered one of the counter-regulator hormones associated with insulin to regulate the secretion of each other, the balance between them is linked with energy metabolism (Huang *et al.* 2020). The correlation between growth hormone and diabetes is a negative significant correlation (-0.726) with a P value at 0.01 level (Table 4.23 and Figure 4.10).

Table 4.22 Growth hormone and diabetes

Group of study	Mean	N	Std. Deviation
Patient	17.7122	90	6.72800
Control	33.7667	60	8.53183
Total	24.1340	150	10.86903

Table 4.23 Correlation and Sig. between growth hormone and diabetes

		Group of study	Growth hormone
Group of study	Pearson-Correlation	1	0.726**
	Sig.(P)		0.000
	No. of sample	150	150
Growth hormone	Pearson-Correlation	0.726**	1
	Sig.(P)	0.000	
	No. of sample	150	150

** Correlation is significant at the 0.01 level (2-tailed).

4.13 Hemoglobin and Diabetes

Table 4.24's data revealed that there is a significant difference in the hemoglobin level between the patient group and the control group, that the patient group had a significantly reduced Hemoglobin concentration when compared to healthy persons, and that the patient group recorded 9.35 g/dL while the control group recorded 11.86 g/dL. According to the findings in Table 4.25, there is a significantly negative connection between hemoglobin and diabetes (-0.756 **), and this correlation is statistically significant at the 0.01 level. These findings were in agreement with the findings of Kwon and Ahn, which included that anemia is relatively speaking common in diabetes patients and that decreased amounts of hemoglobin are linked with a rapid decline in filtration rate compared to other types of renal disease (Kwon and Ahn 2012), whereas Thomas *et al.* found that patients with diabetes were more susceptible to the effects of anemia (Thomas *et al.* 2003). According to research done by Al-Khoury *et. al.*, persons with diabetes have a hemoglobin level that is 1 g/dL lower than those who do not have diabetes.

Table 4.24 Hb and diabetes

Group of study	Mean	N	Std. Deviation
Patient	9.3567	90	.61261
Control	11.8683	60	1.52377
Total	10.3613	150	1.63333

Table 4.25 Correlation and Sig. between Hb and diabetes

		Group of study	Hb
Group of study	Pearson-Correlation	1	0.756**
	Sig.(P)		0.000
	No. of sample	150	150
Hb	Pearson-Correlation	0.756**	1
	Sig.(P)	0.000	
	No. of sample	150	150

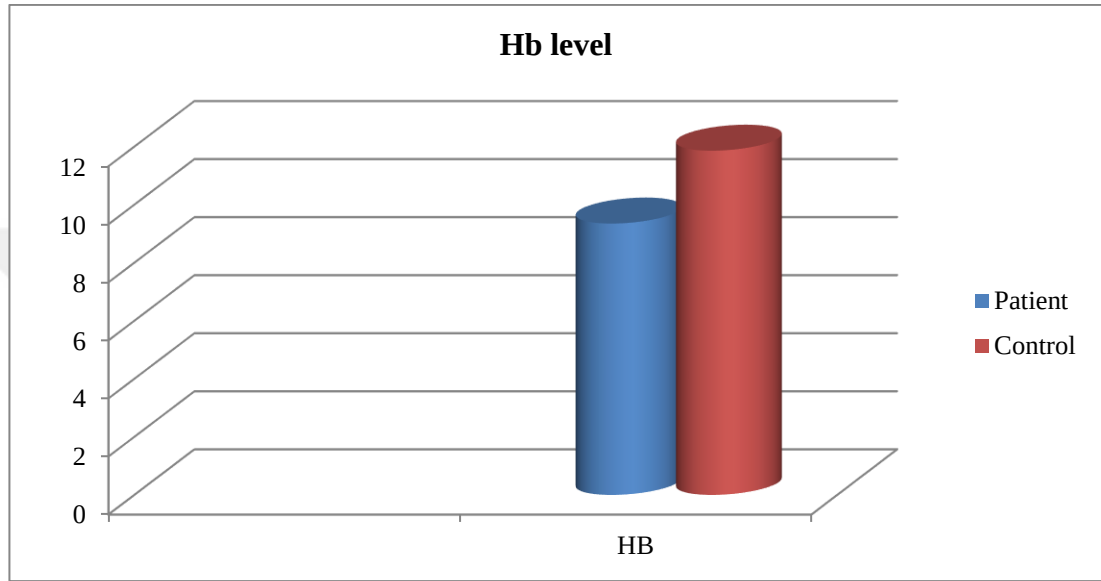


Figure 4.10 Hb level and study groups

4.14 WBC and Diabetes

Results of (Table 4.26) showed that there is a significant difference in WBC count between the two groups, patient group significantly increased the count of WBC as compared with the control group, the patient group recorded 9.65 cells/micron while the control group recorded 7.69 cells/micron, the elevation in WBC count of patients with diabetes may be due to patients with diabetes are more likely to have inflamed blood vessels. These results were in agreement with the results of Martin Jr *et al.*, who found that decreased in diabetes children and highly evaluated in WBC, MPV, and PDW in diabetes type 1 than healthy individuals. Data in (Table 4.27) revealed that there is a negative correlation between WBC and diabetes (Martin Jr *et al.* 2019).

Table 4.26 WBC and diabetes

Group of study	Mean	N	Std. Deviation
Patient	9.6511	90	1.00853
Control	7.6980	60	.52003
Total	8.8699	150	1.27918

Table 4.27 Correlation and Sig. between WBC and diabetes

		Group of study	WBC
Group of study	Pearson-Correlation	1	-0.751**
	Sig.(P)		0.000
	No. of sample	150	150
WBC	Pearson-Correlation	-0.751**	1
	Sig.(P)	0.000	
	No. of sample	150	150

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

The aim of the study is to the estimation of vitamin D and calcium levels in children with and without diabetes disease. Evaluation of some biochemical tests and study the effect of gender and weight on study parameters and study the correlations between these parameters in diabetes children patients' group and healthy individuals control groups.

The results revealed that there is a significant difference in gender parameters of the two study groups (patients group and healthy individuals control group) whereas it seems that the males were more sensitive to diabetes than females, the percentage of males with diabetes disease in patients group and control group recorded (71.11 and 73.33)% respectively as compared to percentage of females (28.88 and 26.66)%.

The results indicated no significant in weight and age of the tested groups, the mean weight for the groups of this study ranged between 24.21 kg inpatient group and 21.15 kg in the control group whereas the mean age of the groups ranged between 7.58 years in the control group to 8.40 years inpatient group.

In children who have type 2 diabetes, a lack of vitamin D is linked to both a reduction in the amount of insulin that is produced and an increase in insulin resistance. It is possible that vitamin D is a healthy predictor of the risk of diabetes in children, and that testing for vitamin D may be used to describe prediabetes in children who also have other risk factors of diabetes.

There is a possibility that the rise in RBC count is connected to hyperglycemia, which is characterized by high blood sugar levels and led to an increase in the number of RBC found in the blood samples of youngsters.

The elevated levels of serum calcium seen in the blood of children who have diabetes have been linked to changes in glucose metabolism as well as an increased chance of developing diabetes. An elevated PTH level causes a rise in calcium levels, which in turn might pose complications for the health of diabetic individuals. Also, the increased likelihood of developing diabetes is linked to an elevated amount of urea in the blood, which may be linked to insulin release from pancreatic beta cells.

5.2 Recommendations

We recommend routine hematological monitoring for patients with diabetes.

These results revealed that the vitamin D and calcium levels in the serum blood of diabetes of children may be considered as an indicator for predication of diabetes patients or in individuals or healthy persons that did not appear any symptoms of diabetes disorders.

The calcium level maybe plays an important role in early detection and afterward choosing the suitable drugs for treating diabetes disease.

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